Original Paper

DOI: 10.22067/veterinary.v8i1.48417

Received: 14 July, 2015
Accepted after revision: 29 November, 2016
Published online: 10 April, 2017

Comparison of application of combination immunomagnetic separation and chromagar salmonella medium with conventional culture method for rapid isolation and detection of Salmonella in bovine diarrheic samples

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Keywords

Salmonella; immunomagnetic separation; CHROMagar *Salmonella* medium

Abstract

Various techniques and culture media were developed for rapid identification of Salmonella serovars. However, there are still problems with their sensitivity and specificity. In an attempt to reduce the time spent to obtain a result and to minimize the problems associated with rapid detection systems such as interference from food ingredients debris, micro flora in feces, and lack of sensitivity, there has been a lot of interest in the development of separation and concentration techniques prior to detection of pathogenic organisms. Various techniques have been utilized for this purpose including: filtration, centrifugation, and lectin-based biosorbents. However, the most successful of the approaches for separation and concentration of target organisms has been the use of Immunomagnetic Separation (IMS). This study was conducted with the objective of comparing the conventional microbiological methods to detect salmonella in diarrheic samples with Immunomagnetic separation combined with chromagar salmonellae medium (IMS-CAS). Of the 400 fecal samples tested by the conventional microbiological and IMS-CAS methods, 33 (8.25%) was culture positive for Salmonella serotypes. The IMS-CAS method gave better results than the conventional microbiological method with less false-positive colonies. Sensitivities for the conventional microbiological method and the IMS-CAS were 100%. The specificity of the IMS-CAS method (99.73%) was significantly higher than that of the conventional microbiological method (94.55%). The use of plating IMS on CAS medium demonstrated high levels of sensitivity and specificity and reduced the time to final identification of Salmonella spp., resulting in substantial cost savings. It can be recommended for the primary isolation of Salmonella spp. from stool specimens.

Abbreviations

CAS: CHROMagar Salmonella
ELISA: Enzyme-Linked Immunosorbent Assay
IMS; Immunomagnetic Separation
Mac. Agar: Macconkey Agar
PCR: Polymerase Chain Reaction
TSI: Triple Sugar Iron
XLD: Xylose Lysine Deoxycholate

IRANIAN JOURNAL OF VETERINARY SCIENCE AND TECHNOLOGY

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Introduction

Salmonellosis is a gastroenteritis caused by different serotypes of Salmonella and it is the most common type of food poisoning in the world. Isolation of the organism by stool culture remains the most reliable method for detection, allowing precise identification of the bacteria and antimicrobial susceptibility testing, both of which are critical for disease control. Conven-tional isolation procedures for the detection of *Salmonella* in feces and food products involve pre-enrichment in non-selective broth, selective enrichment and subculture to differential agar. Subsequent biochemical and serological identification of presumptive Salmonella colonies prolong this technique so that these methods take an average of 4–6 days to be completed for negative and positive samples, respectively (Blackburn, 1993; Fung, 2002; Amani et al., 2015).

A number of rapid methods for the detection of Salmonella in Clinical and food samples have been developed, including electrical techniques, immunoassays and nucleic acid probe analyses. However; there are still problems with their sensitivity and specificity. Immunomag-netic Separation (IMS) technique has been applied to a diverse range of bacteria, viruses and parasites in diagnostic microbiology (Olsvik et al., 1994; Salehi et al., 2007). The use of IMS of Salmonella from samples by plating onto differential agar has been applied to replace the se-lective enrichment phase and as a screening test prior to culture to reduce the detection time of positive samples during the investigation of an outbreak involving a retail premise(Safarik et al., 1995; Coleman et al., 1995b)

The main objective of this study is to compare fast methods for the detection of Salmonella in diarrheic samples using Immunomagnetic Separation followed by culturing in CHROMagar Salmonellae Medium.

Materials and Methods

Samples

In all, 400 bovine diarrhoeic fecal samples from dairy herds were collected. Fecal samples freshly placed into plastic bags and were kept at 4°C and immediately transported to Faculty of Veterinary Medicine, University of Tehran, where they were processed within 48 h of collection. The samples were obtained from dairy herds of Tehran, Golestan and Lorestan provinces in Iran.

Conventional microbiological method

Ten grams of fecal samples were inoculated into 9 ml selenite - cystein broth (Merck KGaA, Darmstadt, Germany) for overnight enrichment at 37°C, and later plated on MacConkey agar (Merck KGaA, Darmstadt, Germany) for primary selection. After 24 h incubation at 37°C, presumptive *Salmonella* isolates were confirmed using conventional biochemical tests [triple sugar iron agar (TSI) and urea] and serotyping of isolates was performed by standard aggluti-nation test using O and H antisera (Bacto-Salmonella O antisera; Difco™; Becton Dickinson and Company, Detroit, MI, USA).

CHROMagar Salmonellae Medium preparation

CHROMagar Salmonellae (CAS), a proprietary product, was provided for evaluation by CHROMagar Microbiology, Paris, France. The medium was supplied as a white powder in pre-weighed batches sufficient for 250 ml and was prepared according to the manufacturer's instructions. Powdered CAS was added to distilled water and was dissolved by slow rotation. When it was dissolved, the medium was boiled with continuous stirring for about 2 min until the complete fusion of the agar grains was detected. After boiling, the medium was swirled gently while cooling to 50°C. Then 20 ml of the medium was dispensed into sterile petri dish-es and it was allowed to solidify and dry with the plates lids kept agar. As indicated by the manufacturer, CAS plates were stored at room temperature in a dark container and were used within a week.

Immunomagnetic Separation (IMS)

IMS was performed according to the manufacturer's instructions using magnetic beads coat-ed with the anti-salmonella antibody (Dynabeads® anti-salmonella, Dynal A.S, Oslo, Nor-way). In brief, one ml of the each sample was added to 20μl of washed Dynabeads® anti-salmonella in 1.5 ml micro centrifuge tube. The tubes were incubated for 15 min at room tem-perature with gentle agitation on a tilt-rotating device. The antibodies coated onto the beads bonded to Salmonella antigen forming the beads – bacteria complex. The bead-bacteria com-plexes were separated using a magnetic particle concentrator (MPC) (Dynal MPC-M; Dynal A.S, Oslo, Norway). After discarding the supernatant, beads with attached bacterial cells were washed two times in 1 ml of washing buffer (PBS pH 7.2, and 0.05% Tween 20). The beads were resuspended in 200 μl of phosphate-buffered saline, cultured on CHROMagar *Salmonella* plates overnight at 37°C and examined for the presence of Salmonella colonies. Serotyping of the presumptive isolates was tested as described previously.

Results

Serotyping

Of the 400 fecal sample tested in this study by conventional microbiological and IMS-CAS methods, 33 (8.25%) were culture positive for *Salmonella* serotypes. Of the 33 different sero-types identified, *Salmonella typhimurium* (6.7%) was the most commonly isolated serotype, followed by *Salmonella dublin* (9.1%), *Salmonella virchow* (6.1%), *Salmonella gloucester* (6.1%), and *Salmonella enteritidis*, *Salmonella georgia*, *Salmonella augustenborg* and *Salmonella lindenburg*, each of them being 3%). The majority of

Table1
Time required for completion of IMS-CAS and conventional culture method for detection of *Salmonella* in bovine diarrheic samples

Detection method	Culture Procedures	Biochemical tests	Total time (hours)
Conventional microbiology	Pre-enrichment (24h) Selective enrichment (24h) Selective culture on agar (24 h)	Biochemical (24h)	4 days
IMS-CAS	IMS (1 h) Plating and incubation (24h)	Biochemical (24h)	49 hours

Salmonella field isolates identified belonged to *Salmonella* serogroup B (72.8%), and 12.1% to *Salmonella* serogroup D. *Salmonella* belonging to serogroup C1 (12.1%) and serogroup C2 (3%) were also identified. Time required for completion of each procedure is shown in Table 1.

Comparison of detection methods

When comparing the two methods (conventional microbiological methods and IMS-CAS), we note that all the samples that have been *Salmonella* positive by microbiological method were also positive by IMS-CAS . No false negatives were obtained from either of the test methods, resulting in 100% sensitivity for the identification of *Salmonella* (Table 2).

Discussion

In an attempt to reduce the length of routine microbiological analysis and to minimize the problems associated with rapid detection systems such as interference from feces, foods and food ingredient debris, background micro-organisms, and lack of sensitivity, there has been a lot of interest in the development of separation and concentration techniques prior to detection. Various techniques have been utilized for this purpose including: centrifugation, filtration, and lectin-based biosorbents (Odumeru and Leon-Velarde, 2012). However, the most successful of the approaches for the separation and concentration of target organisms has been the use of Immunomagnetic Separation. The advantages of IMS are that it reduces the total analysis time and improves the sensitivity of detection. IMS is rapid, technically simple, and a specific method for the isolation of the target organisms (Show et al., 1998). Paramagnetic particlesare coated with antibodies specific to the target organism and added to a post enrichment culture. The target organism is captured onto the magnetic particles and the whole complex is then removed from the system by the application of a magnetic field. Target organisms are thus removed from food debris and competing microorganisms, which may otherwise interfere with the detection system. If required, the isolated complex may be re-suspended in an enrichment broth so that cell numbers can be rapidly increased to improve the sensitivity of detection assays. In addition, IMS by design can be used in conjunction with other rapid detection methods, including plating to differential agar, ELISA, conductance microbiology, electrochemiluminescence, and polymerase chain reaction (PCR) to further increase its analytical sensitivity (Yang and Li, 2006; Salehi et al., 2007; Taha et al., 2010; Koluman et al., 2012; Brandao et al., 2013; Zheng et al., 2014). It has been reported that IMS is more sensitive than conventional culture methods and is able to reduce the total culture analysis time by one to two days (Taha et al., 2010; Chao-Yu et al., 2014; Fengying et al., 2015).

In comparison with conventional cultural methods, the IMS-CAS is a rapid, specific method for the detection of *Salmonella* in diarrheic samples that contain a high amount of other mi-croorganisms. The shortened protocols for *Salmonella spp.* detection described here can offer considerable improvement over current methodologies. Separation

Table 2
Comparison IMS-CAS and conventional culture method for specific identification of Salmonella in bovine diarrheic samples

Method	No. of samples	No. of isolates with true positive results	No. of isolates with true negative results	No. of isolates with false positive results	No. of isolates with false negative results	Sensitivity (100 %)	Specificity (100 %)
Conventional microbiology	400	33	347	20	0	100	94.55
IMS+CAS	400	33	366	1	0	100	99.73

of target organisms follow-ing use of IMS is considerably quicker than using selective enrichment and may assist in the recovery of injured cells (Blackburn et al., 1993; Odumeru and Leon-Velarde, 2012; Amani, et al., 2015). When combined with plating to differential agar, Dynabeads anti-*Salmonella* have been shown to give 100% correlation with conventional culture for the detection of *Salmonella* in a variety of food, feed and environmental samples (Shaw et al., 1998; Maddocks et al., 2002). Improved isolation rates have been described when using IMS in the isolation of salmonellae from raw chicken carcasses (Coleman et al., 1995a; Conceicao et al.,2008; Rita de Cássia et al., 2008), and skimmed milk powder (Dziadkowiec et al., 1995).

Compared to the number of false-positive colonies on other selective media such as Mac Conkey agar, Hektoen agar, *Salmonella-Shigella* agar and XLD agar we observed far fewer false-positive colonies on CAS, and all of them could be ruled out as *Salmonella spp.* Fur-thermore, the good sensitivity of CAS qualifies this medium for use in the primary plating of stool specimens when searching for *Salmonella spp.*

Considering our results, we feel that the use of IMS-CAS provides a time-saving method for the detection and presumptive identification of *salmonella* in the routine analysis of stool specimens. Interpretation of colors is easy, and all colonies of *salmonella* tested displayed the same color and morphology.

Acknowledgments

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DOI: 10.22067/veterinary.v8i1.48417

مقایسه کاربرد تؤام جداسازی ایمیونومگنتیک و کروم آگار سالمونلا با کشت متداول برای جداسازی و تشخیص سریع سالمونلا در نمونه‌های اسهالی گاو

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دریافت مقاله: ۱۳۹۴/۰۴/۲۳ پذیرش نهایی ۱۳۹۵/۰۹/۰۹

چکیده

تکنیک‌ها و محیط‌های کشت متنوعی برای تشخیص سریع سرووارهای سالمونلا توسعه یافته است. با این همه هنوز مشکلات حساسیت و ویژگی‌های آنها پابرجا است. به منظور کاهش زمان اخذ نتیجه و به حداقل رساندن مشکلات مرتبط با روش‌های سریع نظیر تداخل بقایای مواد غذایی و میکروفلورای مدفوع و نیز فقدان حساسیت، توجه زیادی به توسعه روش‌های جداسازی و تغلیظ ارگانیسم‌های بیماریزا قبل از تشخیص معطوف گشته است. روش‌های متنوعی نظیر فیلتراسیون، سانتریفیوژ و بیوسوربنت‌های لکتینی استفاده شده است، لیکن موفق‌ترین روش برای این منظور روش ایمیونومگنتیک بوده است. این بررسی با هدف مقایسه روش کشت متداول جهت شناسایی سالمونلا در نمونه های اسهالی گاو با روش تؤام ایمیونومگنتیک و محیط کروم آگار سالمونلا (IMS-CAS) انجام گرفت. از ۴۰۰ نمونه اسهالی که با هر دو روش کشت متداول میکروبی وIMS-CAS مورد آزمایش قرار گرفت، ۳۳ نمونه (۸/۲۵ درصد) برای سالمونلا مثبت بودند. روش IMS-CAS نتایج بهتر و پرگنه‌های مثبت کاذب کمتری نشان داد. حساسیت هر دو روش کشت متداول میکروبی و IMS-CAS ۱۰۰ درصد بود. ویژگی روش IMS-CAS (۹۹/۷۳ درصد) بطور معنی داری بالاتر از روش کشت متداول میکروبی (۹۴/۵۵ درصد) بود. نتایج نشان داد که استفاده از تکنیک ایمیونومگنتیک و کشت آن بر روی محیط کروم آگار سالمونلا درجه بالایی از حساسیت و ویژگی نشان داده و زمان تشخیص سالمونلا را کاهش و در نتیجه کاهش هزینه‌ها می شود. روش ایمیونومگنتیک برای جداسازی اولیه سالمونلاها در نمونه های مدفوعی توصیه می شود.

واژگان کلیدی: سالمونلا، جداسازی ایمیونومگنتیک، کروم آگار سالمونلا